REVIEW

The long ($G_{s\alpha\text{-}L}$) and short ($G_{s\alpha\text{-}S}$) variants of the stimulatory guanine nucleotide-binding protein. Do they behave in an identical way?

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ABSTRACT

The relative proportions and tissue distribution of the long ($G_{s\alpha\text{-}L}$) and short ($G_{s\alpha\text{-}S}$) variants of the α subunit of the stimulatory G-protein ($G_{s\alpha}$) change under a wide range of metabolic conditions, such as cellular differentiation, ontogenetic development, ageing and various adaptive processes. Although the two variants of $G_{s\alpha}$ are generally regarded to be functionally identical, this review summarizes recent experimental support for the non-identical behaviour of these proteins. Similarly, there is no consistent evidence for the functional meaning of these changes as far as regulation of adenylate cyclase activity is concerned. Since it is hard to believe that the complicated scheme of alternative splicing and the energy-consuming synthesis of proteins would be performed for no reason, it is suggested that $G_{s\alpha}$ variants might be involved in controlling other effector molecules and processes besides adenylate cyclase and cAMP metabolism. Such an idea is indirectly supported by specific alterations in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio under various physiological and pathophysiological conditions.
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INTRODUCTION

Cell responses to a wide variety of extracellular signals are mediated by heterotrimeric guanine nucleotide-binding proteins (G-proteins), which are composed of α, β and γ subunits. The α subunit is responsible for specific interactions with both the receptor and effector molecules and it primarily determines the function of a G-protein (Gilman 1987, Helmreich & Hofmann 1996). In this review we focus our attention on the properties and behaviour of the individual variants (subforms, isoforms, species) of the α subunits of the stimulatory G-protein ($G_{s\alpha}$), which have been established as the stimulatory regulatory components of adenylate cyclase and may also be involved in activation of dihydropyridine-sensitive voltage-gated Ca^{2+} channels in skeletal muscle and inactivation of cardiac Na^{+} channels (Mattera *et al.* 1989, Schubert *et al.* 1989, Birnbaumer *et al.* 1990). As for other G-proteins, the molecular interactions responsible for G_s-modulated transmembrane signalling are driven by a cycle of guanine nucleotide exchange and hydrolysis (Fig. 1).

Two forms of $G_{s\alpha}$ ubiquitously expressed in various tissues have been identified by their ability to be ADP-ribosylated by cholera toxin (Northup *et al.* 1980) or by immunoblotting with specific antibodies (Mumby *et al.* 1986). These proteins have been reported to migrate in polyacrylamide gels with apparent M_r values of 52 000 and 45 000 by some authors (Jones & Reed 1987, Feldman *et al.* 1990, Sethi *et al.* 1993) or 45 000 and 42 000 by others (Scherer *et al.* 1987, Milligan 1990, McFarlane-Anderson *et al.* 1992, Negishi *et al.* 1992), depending on the materials and experimental conditions used. Both the long and short forms of $G_{s\alpha}$ ($G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$) have been shown to be produced by alternative splicing of a single pre-mRNA transcript (Robishaw *et al.* 1986, Kozasa *et al.* 1988). The human $G_{s\alpha}$ gene is a split gene composed of 13 exons and 12 introns that span a region of about 20 kb of genomic DNA (Kozasa *et al.* 1988). The long and short forms of $G_{s\alpha}$ differ by 14 or 15 amino

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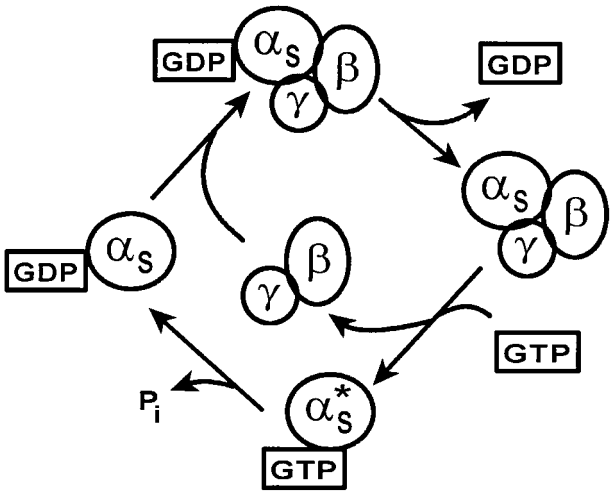

FIGURE 1. A simplified scheme of the G_s regulatory cycle. Activation of G_s is substantiated by exchange of GTP for GDP on α_s. The transition from inactive ($\alpha_{s(GDP)}$) to active ($\alpha_s{}^*{}_{(GTP)}$) conformation is accelerated by interaction of G_s with a cognate ligand-activated receptor, which stimulates GDP release and facilitates GTP binding. Upon activation, the $\alpha_s{}^*\beta\gamma$ complex dissociates and both free activated $G_{s\alpha}$ and $G_{\beta\gamma}$ can interact with appropriate effectors. Hydrolysis of GTP by $G_{s\alpha}$ deactivates it, increases its affinity for $G_{\beta\gamma}$, and leads to reassociation to give an inactive GDP-bound G_s.

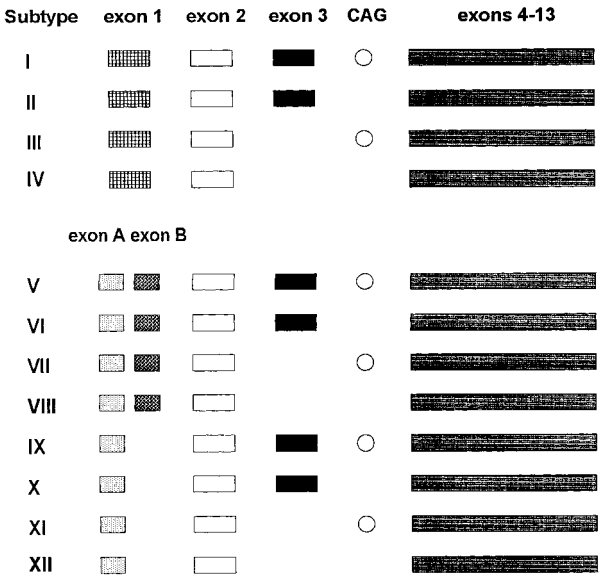

FIGURE 2. Origin of possible subtypes of $G_{s\alpha}$ cDNAs by alternative splicing of 5′-exons (modified from Swaroop *et al.* 1991). The first four $G_{s\alpha}$ cDNA species (I–IV) differ from each other by the presence of an exon 3 and/or a CAG trinucleotide at the boundary of exons 3 and 4. The next eight variants (V-XII) contain novel exons upstream of exon 2 (exon A and/or B) and they may be generated either by alternative splicing or by using an alternative promoter. In addition, aberrant splicing events involving internal deletions at non-consensus sites have been also described (Ali *et al.* 1992), and Crawford *et al.* (1993) reported neural expression of the $G_{s\alpha}$N1 transcript generated by alternative splicing and polyadenylation of a novel terminal exon downstream of exon 3.

acid residues, which are coded by exon 3 (Fig. 2). Originally it was thought that there were four splicing products (I–IV) of a single $G_{s\alpha}$ gene, resulting from alternative use of exon 3 and of two 3′ splice sites of intron 3 (Bray *et al.* 1986), but several additional splicing variants of $G_{s\alpha}$ have since been described (Ishikawa *et al.* 1990, Swaroop *et al.* 1991, Ali *et al.* 1992, Crawford *et al.* 1993, Habecker *et al.* 1993). It is not known if all splicing variants are expressed as mature proteins. Some of these couple receptors to stimulation of adenylate cyclase and Ca^{2+} channels, whereas others encode truncated proteins the functions of which are not currently defined.

Besides typically ubiquitous long and short forms of $G_{s\alpha}$, there exists a closely related α subunit of the olfactory GTP-binding protein ($G_{\alpha olf}$, M_r ~42 000), which is distributed in vertebrate chemosensory neurons only (Pace & Lancet 1986, Jones & Reed 1987, 1989, Jones *et al.* 1990). Two forms of $G_{s\alpha}$-like subunit (M_r ~50 000 and 45 000) have been described in *Drosophila melanogaster*, which function in a manner similar to their mammalian homologues (Quan *et al.* 1991), and another functional $G_{s\alpha}$-like protein (M_r ~42 000) has recently been identified in the free-living protist *Euglena gracilis* (Torresmarquez *et al.* 1996). In addition, a tightly membrane-associated 'extra large' G-protein, XLαs (M_r ~94 000), has been revealed in the *trans*-Golgi network, which consists of a new 51 kDa XL-portion linked to a $G_{s\alpha}$ truncated at the N-terminus (Kehlenbach *et al.* 1994).

During the last decade, a large amount of information has been gathered about the unequal distribution of $G_{s\alpha}$ variants in various tissues. To give some examples, $G_{s\alpha\text{-}L}$ markedly predominates in kidney, placenta, adrenal medulla, cortex and cerebellum (Evans *et al.* 1986, Mumby *et al.* 1986, Granneman & Kapatos 1990, Feinstein *et al.* 1992, Michel *et al.* 1994), whereas $G_{s\alpha\text{-}S}$ is the prevailing form in heart, liver, neostriatum and platelets (Mumby *et al.* 1986, Molina y Vedia *et al.* 1989, Cooper *et al.* 1990, Kawai & Arinze 1996). The existence of different forms of $G_{s\alpha}$ with varying tissue distribution invites speculation that they may have evolved to perform different regulatory functions. To date, however, there is only a small

amount of evidence to indicate that the $G_{s\alpha}$ variants have more than marginal differences in properties and function.

$G_{s\alpha\text{-}L}$ AND $G_{s\alpha\text{-}S}$ ARE FUNCTIONALLY IDENTICAL

Studies with purified $G_{s\alpha}$ produced in bacteria have shown only minor functional differences between $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ (Graziano *et al.* 1987, 1989, Mattera *et al.* 1989, Freissmuth 1991). Recombinant $G_{s\alpha}$ proteins interact functionally with $G_{\beta\gamma}$, β-adrenergic receptors, adenylate cyclase and Ca^{2+} channels and they can be equally ADP-ribosylated by cholera toxin. Both forms of recombinant $G_{s\alpha}$ hydrolyse GTP and have essentially the same k_{cat} for GTP hydrolysis (about $4\ min^{-1}$). The only difference between bacterially expressed $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ is a modest difference in the rate of GDP dissociation, otherwise they are biochemically indistinguishable in *in vitro* reconstitution assays. Further experiments revealed that the affinity of recombinant $G_{s\alpha}$ for adenylate cyclase is roughly 10 times lower than that of native $G_{s\alpha}$ purified from the liver, while the intrinsic capacity of the recombinant protein to activate adenylate cyclase is normal (Graziano *et al.* 1989). This can probably be seen as a consequence of the failure of $G_{s\alpha}$ to undergo a post-translational modification that is essential to achieve high-affinity interaction of the G-protein with adenylate cyclase.

Studies using $G_{s\alpha}$ expression in mammalian systems have provided a similar picture. No substantial difference has been found in the ability of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ expressed in S49 cyc^- cells to stimulate adenylate cyclase activity and to couple to β-adrenergic receptors (Jones *et al.* 1990, O'Donnell *et al.* 1991). In another study, the region of variation between the long and short variants of $G_{s\alpha}$ was modified by genetic engineering to produce an epitope-tagged variant of $G_{s\alpha\text{-}L}$ (Levis & Bourne 1992). The cellular distribution (membrane-bound vs soluble form) of tagged and untagged $G_{s\alpha}$ constructs expressed in S49 cyc^- cells was identical, suggesting that the epitope does not disturb targeting of the protein to the plasma membrane, and no significant alterations were observed in the method of regulation of adenylate cyclase by these two variants.

$G_{s\alpha\text{-}L}$ AND $G_{s\alpha\text{-}S}$ ARE NOT FUNCTIONALLY IDENTICAL

The first evidence in favour of the idea that the two variants of $G_{s\alpha}$ are not identical from a functional point of view was put forward by Sternweis *et al.* (1981), who, by testing partially purified preparations of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ from rabbit liver, found that the larger species has a greater ability to support hormone-stimulated adenylate cyclase activity. Similar results have been reported by Yagami (1995), who studied the interaction of $G_{s\alpha}$ subforms with β-adrenergic and glucagon receptors in liver plasma membranes. It has been shown that activated glucagon receptors, by accelerating the rate of exchange of bound GDP for GTP on $G_{s\alpha}$, enhance substantially the sensitivity of both $G_{s\alpha}$ variants to tryptic digestion, while activated β-adrenergic receptors have a similar effect on $G_{s\alpha\text{-}L}$ only. This observation supports the notion that β-adrenergic receptors are preferentially coupled to the long form of $G_{s\alpha}$.

In contradiction, a higher capability of the short isoform of $G_{s\alpha}$ to transmit signals has been suggested by Walseth *et al.* (1989). This departure from the commonly held view that the two subunits have similar efficacies in stimulating adenylate cyclase was based on the increase in responsiveness to isoprenaline, cholera toxin and forskolin in master pancreatic islet β cells as a function of passage number. The increased number of passages in the course of cultivation of these cells was accompanied by a decrease in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio from 1·0 to 0·22, and the extracts derived from later passages reconstituted adenylate cyclase activity in S49 cyc^- membranes 3-4 times more effectively.

The idea of functional 'non-identity' of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ has also been supported by the different behaviour of the two isoforms in the course of subcellular redistribution of the $G_{s\alpha}$ proteins induced by prolonged agonist (isoprenaline) stimulation of S49 lymphoma cells (Kvapil *et al.* 1994). The transfer of $G_{s\alpha}$ functional activity (measured by a cyc^- reconstitution assay) from plasma membranes to low-density membranes (light vesicles) was accompanied by an increase in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio in the latter membrane fraction. In addition, the light-vesicular fractions enriched in $G_{s\alpha\text{-}L}$ proved to be more effective in protecting adenylate cyclase against its thermal inactivation (Kvapil *et al.* 1995*a*). This observation may indicate different quality of the interaction between adenylate cyclase and the long and short $G_{s\alpha}$ subforms. Similar subcellular redistribution of $G_{s\alpha}$ has been recently described as a consequence of long-term forskolin treatment of astroglial cells (El Jamali *et al.* 1996). In membrane preparations from placental vascular smooth muscle, vasoactive intestinal peptide in the presence of guanosine 5′-[γ-thio]triphosphate (GTPγS) also triggered a dose-dependent release of both $G_{s\alpha}$ subforms into the cytosolic fraction, while

the amount of $G_{s\alpha\text{-}S}$ released appeared to be much higher than that of $G_{s\alpha\text{-}L}$ (Bourgeois *et al*. 1996).

An interesting difference in the behaviour of the $G_{s\alpha}$ variants was also observed in experiments performed with reconstituted phospholipid vesicles, where different recoveries (different molar ratios of α to $\beta\gamma$) for $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ were determined for different preparations of G-protein $\beta\gamma$ subunits (Rubenstein *et al*. 1991). Two preparations of $\beta\gamma$ subunits differed reproducibly in their ability to efficiently reconstitute $G_{s\alpha\text{-}S}$, while no substantial change was detected with respect to $G_{s\alpha\text{-}L}$. These results indicate for the first time that different combinations of β and γ subtypes can interact unequally with the long and short $G_{s\alpha}$ subforms.

IN VITRO REGULATION OF $G_{s\alpha\text{-}L}$ AND $G_{s\alpha\text{-}S}$: STUDIES ON TISSUE CULTURES

Maintained exposure of NG108-15 cells (neuroblastoma–glioma hybrid) to agonists at the prostanoid IP receptor results in substantial reduction (down-regulation) of total cellular levels of $G_{s\alpha}$ (McKenzie & Milligan 1990, Adie *et al*. 1992). Agonist-induced down-regulation of $G_{s\alpha}$ protein levels is dependent on the level of receptor expression and is generally restricted to the G-protein(s) with which the receptor interacts (Milligan 1993, Adie & Milligan 1994). Alterations in the individual variants of $G_{s\alpha}$, however, have not been studied in this respect and it remains to be clarified whether $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ respond to long-term agonist treatment in the same way.

Foster *et al*. (1990) have studied the effect of K^+-induced depolarization on G-protein expression in spontaneously contracting neonatal rat myocytes. They observed that within 3 days of cultivation in medium containing high concentrations of KCl, the cells began to accumulate $G_{s\alpha\text{-}S}$, which was virtually not expressed under control conditions. The level of $G_{s\alpha\text{-}L}$ in myocyte membranes was not substantially influenced and the changes in $G_{s\alpha\text{-}S}$ induced by KCl depolarization appeared to be reversible.

When selective patterns of expression of G-protein α subunits during *in vitro* development of primary hypothalamic neuron-enriched cultures was studied, no $G_{s\alpha\text{-}S}$ was detected (Viollet *et al*. 1994). Nevertheless, remarkably dynamic short-term changes in $G_{s\alpha}$ mRNA as well as $G_{s\alpha\text{-}L}$ protein levels were demonstrated. While the cellular concentration of $G_{s\alpha}$ mRNA increased by roughly 75% after 5 days and this level was maintained for the next 2 weeks of cultivation, $G_{s\alpha\text{-}L}$ immunoreactivity doubled during the first 5 days of development and then fell sharply. Thus the time-related decrease in $G_{s\alpha}$ protein level was not parallelled by the corresponding mRNA. Similarly, rapid disappearance of $G_{s\alpha}$ proteins has been reported by Cussac *et al*. (1990) in mouse hypothalamus shortly after birth and in fetal hypothalamic cells cultivated *in vitro* (Kitamura *et al*. 1989).

Alterations in the relative amounts of $G_{s\alpha}$ variants have also been observed during maturation of reticulocytes. Although the concentration of both isoforms of $G_{s\alpha}$ decreased with maturation, a greater reduction in $G_{s\alpha\text{-}L}$ was detected (Larner & Ross 1981).

When characterizing the potential roles of G_α proteins in differentiation of white adipocytes cultivated in primary culture, Denis-Henriot *et al*. (1996) detected mainly $G_{s\alpha\text{-}L}$ in confluent preadipocytes, whereas mature adipocytes expressed both the long and short variants. Differential expression of the $G_{s\alpha}$ isoforms has also been found during differentiation of 3T3-L1 fibroblasts, which can readily differentiate into cells possessing the morphological and biochemical properties of adipocytes (Watkins *et al*. 1982, 1987, 1989). In 3T3-F442 fibroblasts, the levels of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ increased during differentiation by about 125% and 750% respectively (Kilgour & Anderson 1993).

IN VIVO REGULATION OF $G_{s\alpha\text{-}L}$ AND $G_{s\alpha\text{-}S}$

Cardiovascular system

Under 'more physiological' conditions, non-identical behaviour (regulation) of the two $G_{s\alpha}$ variants has also been demonstrated. Significant alterations have been determined during ontogenetic development and ageing. The amounts of $G_{s\alpha\text{-}L}$ in rabbit heart muscle do not change during the first 6 weeks of life, while those of $G_{s\alpha\text{-}S}$ substantially increase during this period of postnatal development (Kawai & Arinze 1996). Interestingly, the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio (about 0·4) in plasma membranes derived from rabbit liver remained unchanged during the first 6 weeks of life, but the total amount of $G_{s\alpha}$ was markedly elevated in 6-week-old animals (Kawai & Arinze 1991).

The opposite type of alteration has been described in rat hearts during ageing. A significant decrease in $G_{s\alpha\text{-}S}$ parallelled by an increase in $G_{s\alpha\text{-}L}$ was determined by cholera toxin-induced ADP ribosylation in samples prepared from 24-month-old rats, compared with 6-month-old animals (Urasawa *et al*. 1991). Similar but less pronounced age-related changes in the distribution of $G_{s\alpha}$ variants in rat heart have been shown by

immunodetection techniques (Shu & Scarpace 1994). Interestingly, basal, isoprenaline- and forskolin-stimulated adenylate cyclase activity in myocardial membranes decreased during ageing, whereas adenylate cyclase activity stimulated by the non-hydrolysable GTP analogues GTPγS and guanosine 5′-[β,γ-imido]triphosphate (p[NH]ppG) was significantly potentiated. An age-related decline in $G_{s\alpha}$ gene transcripts, which was associated with down-regulation of all the $G_{s\alpha}$ protein species, has been reported by Miyamoto *et al*. (1994) in rat ventricular myocardium. When studying expression of G-proteins in cardiocytes, Foster *et al*. (1990) observed that neonatal rat cardiac myocytes and non-muscle heart cells contained preferentially $G_{s\alpha\text{-}L}$ and almost no $G_{s\alpha\text{-}S}$. In contrast, atrial membranes from adult rat hearts had approximately equal amounts of the two $G_{s\alpha}$ variants, and adult ventricles had predominantly $G_{s\alpha\text{-}S}$.

In the aorta of adult and senescent rats, no significant difference has been detected in the levels of $G_{s\alpha}$ mRNAs (Johnson *et al*. 1995), while an immunoblot analysis using specific $G_{s\alpha}$-oriented antibodies revealed a significant age-related decrease in the content of the $G_{s\alpha\text{-}S}$ protein (but not $G_{s\alpha\text{-}L}$) in 24-month-old rats, compared with 6-month-old animals.

Kidney

Marked alterations in the relative proportion of $G_{s\alpha}$ variants have also been found in rat kidney during ontogenesis. While the levels of $G_{s\alpha\text{-}L}$ in membrane preparations prepared from 3-week-old and 28-week-old rats were roughly identical, the content of $G_{s\alpha\text{-}S}$ doubled in preparations from older animals (Michel *et al*. 1994). Almost the opposite phenomenon has been observed in age-matched spontaneously hypersensitive rats; $G_{s\alpha\text{-}S}$ did not change, whereas $G_{s\alpha\text{-}L}$ significantly decreased in membranes derived from 28-week-old rats (Michel *et al*. 1994). The reduced $G_{s\alpha\text{-}L}$ content may well be the molecular basis for the previously observed unchanged isoprenaline-stimulated cAMP formation despite the increased β-adrenergic receptor number (Michel *et al*. 1993).

Liver

The effect of partial hepatectomy on the levels of $G_{s\alpha}$ variants in rat liver plasma membranes has been studied by Yagami *et al*. (1994). The amounts of both $G_{s\alpha}$ species increased after hepatectomy, with a maximum at 48-72 h, and subsequently decreased. The increase in $G_{s\alpha\text{-}L}$ was more significant than that in $G_{s\alpha\text{-}S}$. The maximal amounts of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ were 2·4-fold and 1·8-fold higher respectively when compared with samples from animals before sham operation. The abundance of the $G_{s\alpha}$ mRNAs reached a maximum at 24-48 h, and the increase in $G_{s\alpha\text{-}L}$ mRNA was higher than that in $G_{s\alpha\text{-}S}$ mRNA, which may well reflect the differences in the elevated levels of $G_{s\alpha}$ proteins. Perhaps it is not such a surprise that the time courses of quantitative changes in $G_{s\alpha}$ proteins were accompanied by corresponding changes in catecholamine-responsive adenylate cyclase activity.

When studying the adenylate cyclase signalling system in liver plasma membranes from lean and genetically diabetic (*db*/*db*) mice, Palmer & Houslay (1991) noted that treatment of animals with pertussis toxin significantly influenced levels of $G_{s\alpha}$. In membranes prepared from pertussis toxin-treated lean rats, the expression of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ was increased 2·5-fold and 2·9-fold respectively, whereas the levels of these variants in membranes from diabetic animals were reduced by 20% and 15% respectively. A similar enhancement of the ability of glucagon, isoprenaline and p[NH]ppG to activate adenylate cyclase was observed in samples from both lean and diabetic rats. It therefore seems likely that *in vivo* treatment of rats with pertussis toxin elicits alterations extending far beyond the simple modification of $G_{i\alpha}$ by ADP-ribosylation. Nevertheless, the reason for its opposing effects on the expression of $G_{s\alpha}$ variants in lean and diabetic animals is unclear.

Brain

The ratios of $G_{s\alpha}$ variants in mouse brain change dramatically during development. The $G_{s\alpha\text{-}L}$ content was found to be more than twice that of the adult in the embryo, whereas $G_{s\alpha\text{-}S}$ represented no more than 10% of the level determined in adult animals (Rius *et al*. 1991). The amount of $G_{s\alpha\text{-}L}$ decreased as $G_{s\alpha\text{-}S}$ increased during the postnatal period and at day 14 the concentrations of the long and short $G_{s\alpha}$ isoforms in whole brain membranes were nearly equal. These changes were accompanied by a significantly enhanced sensitivity of adenylate cyclase to stimulation by p[NH]ppG. The reciprocal relationship between the two isoforms of $G_{s\alpha}$ suggests that the alternative splicing mechanism of $G_{s\alpha}$ might be involved in ontogenetic development of brain tissue.

In rat telencephalon, a gradually increasing concentration of $G_{s\alpha\text{-}L}$ has been detected during the first few days after birth by Kitamura *et al*. (1989). After reaching its peak level at day 12, $G_{s\alpha}$ decreased to the fetal level, which was then maintained at the adult stage.

Differential expression of $G_{s\alpha}$ variants has also been described in the cerebellum and neostriatum, in which $G_{s\alpha\text{-}L}$ (cerebellum) and $G_{s\alpha\text{-}S}$ (neostriatum) are predominantly expressed (Cooper *et al*. 1990). Since neostriatum is far less rich in Ca^{2+}/calmodulin-regulated adenylate cyclase than most brain regions (including cerebellum), it can be hypothesized that preferential expression of $G_{s\alpha\text{-}S}$ might be selectively associated with Ca^{2+}/calmodulin-independent types of adenylate cyclase.

Ozawa *et al*. (1993) have found a reduction in the concentration of $G_{s\alpha\text{-}L}$ but not $G_{s\alpha\text{-}S}$ in synaptic membranes derived from the temporal cortex of alcoholics. A decrease in $G_{s\alpha}$ in anterior pituitary and cerebellar membranes has also been reported after chronic ethanol administration to long-sleep mice (Wand & Levine 1991). Immunoblotting techniques have revealed different distributions of $G_{s\alpha}$ variants in post-mortem samples of frontal cortex of suicide victims (Cowburn *et al*. 1994). The $G_{s\alpha\text{-}S}$ content showed a tendency to be increased in the both violent death and depressed suicide subgroups. The levels of $G_{s\alpha\text{-}L}$ exhibited a significant positive correlation (increase) with the age of the experimental subject. This observation is in contrast with that reported by Young *et al*. (1991*a*) for human parietal cortex, where a strikingly higher ratio of $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ was found in infants than in adults. This resulted from lower amounts of $G_{s\alpha\text{-}L}$ together with much higher concentrations of $G_{s\alpha\text{-}S}$ in adulthood. Patients with bipolar affective disorder exhibited an increased $G_{s\alpha}$-related immunoreactivity in cerebral cortex, which was mainly due to an increased concentration of $G_{s\alpha\text{-}L}$ compared with that in age- and sex-matched controls (Young *et al*. 1991*b*).

In brain membranes of genetically obese (*ob*/*ob*) mice, the total amount of immunochemically detectable $G_{s\alpha}$ was increased and the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio shifted from 1·64 to 2·8, compared with lean controls (McFarlane-Anderson *et al*. 1992). Interestingly, liver membranes from the same obese mice contained roughly half the amount of the $G_{s\alpha}$ determined in lean controls, and the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio remained almost unchanged (McFarlane-Anderson *et al*. 1992).

Myometrium

Different patterns of expression of $G_{s\alpha}$ mRNA splice variants as well as $G_{s\alpha}$ proteins have been observed in human myometrium during gestation (Europe-Finner *et al*. 1993, 1994, 1996). The $G_{s\alpha}$ levels were considerably higher in myometrium taken from pregnant than from non-pregnant women, while the increase in $G_{s\alpha\text{-}L}$ was more pronounced than that in $G_{s\alpha\text{-}S}$. Interestingly enough, the amounts of both $G_{s\alpha}$ species returned to control levels at the spontaneous onset of term or preterm labour.

White and brown adipose tissue

Specific age-related changes in the long and short isoforms of $G_{s\alpha}$ have been found in epididymal white adipose tissue. Adipocyte membranes isolated from 18-month-old rats exhibited a 2-fold increase in $G_{s\alpha\text{-}S}$ as compared with 9-week-old animals (Green & Johnson 1989). No change was detected in the content of $G_{s\alpha\text{-}L}$. Similar results were obtained by both immunoblotting and cholera toxin-induced ADP-ribosylation.

Quantification of $G_{s\alpha}$ variants by immunodetection in white adipose tissue of genetically diabetic (*db*/*db*) mice revealed that the long isoform was significantly less abundant than in non-diabetic controls, whereas no difference was detected in the amount of the short isoform (Begin-Heick 1992, 1996). A similar result was obtained for genetically obese (*ob*/*ob*) mice as compared with lean littermates (Begin-Heick 1990). An identical reduction in $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ levels was found by Strassheim *et al*. (1991) in the adipocytes of obese (*fa*/*fa*) Zucker rats. This reduction in $G_{s\alpha}$ proteins was accompanied by attenuated activation of adenylate cyclase by stimulatory ligands.

Another type of evidence in favour of differential regulation of the steady-state levels of the short and long variants of $G_{s\alpha}$ was obtained in studies of brown adipose tissue. The perinatal stimulation (recruitment) of brown fat was associated with changes in the splicing pattern of $G_{s\alpha}$ mRNA, and these changes were reflected in $G_{s\alpha}$ protein expression (Granneman *et al*. 1990). $G_{s\alpha\text{-}L}$ mRNA increased significantly without any effect on the level of $G_{s\alpha\text{-}L}$ protein, whereas $G_{s\alpha\text{-}S}$ mRNA did not change and $G_{s\alpha\text{-}S}$ protein concentration declined to 40% of the control level. The increase in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio was therefore achieved by the preferential decrease in $G_{s\alpha\text{-}S}$, with little or no change in $G_{s\alpha\text{-}L}$ protein (Chaudhry & Granneman 1991).

Granneman & Bannon (1989) and Granneman *et al*. (1990) also suggested that the increased $G_{s\alpha}$ mRNA levels that occur in brown adipose tissue during periods of stimulation serve to maintain membrane levels of $G_{s\alpha}$ protein. The reason why this is needed may well involve the agonist-induced down-regulation of the $G_{s\alpha}$ subunits, which proceeds primarily as increased proteolytic degradation of this protein (McKenzie & Milligan 1990, Milligan 1993, Mitchell *et al*. 1993).

A similar change in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio has been found in another 'recruited' state of brown adipose tissue metabolism, the one induced by long-term adaptation to cold (cold acclimation). Cold acclimation was found to be associated with a preferential decrease in $G_{s\alpha\text{-}S}$ in brown adipose tissue membranes, whereas no change was detected in the amount of $G_{s\alpha\text{-}L}$ (Kvapil *et al.* 1995*b*). This result was based on resolution of plasma membrane proteins by standard SDS–PAGE and immunoblot analysis with antiserum raised against an internal sequence (amino acids 326-335) of $G_{s\alpha}$. When another type of antiserum (raised against the C-terminal decapeptide of $G_{s\alpha}$) and high-resolution urea–SDS–PAGE was used, the results were very similar: cold acclimation was associated with an increase in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio from 2·39 to 3·03 as compared with controls (P Svoboda, L Bourova & J Novotny, unpublished results).

Thus the two types of recruitment of brown adipose tissue, i.e. cold acclimation and perinatal cold stress, seem to be associated with similar changes in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio. The increase in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio induced by perinatal cold stress, however, is accompanied by an increase in noradrenaline- and fluoride-stimulated adenylate cyclase (Chaudhry & Granneman 1991) while the isoprenaline-, fluoride- and GTPγS-stimulated adenylate cyclase activity in plasma membranes isolated from cold-acclimated animals is decreased; the same applies to the functional activity of $G_{s\alpha}$ assessed by a cyc^- reconstitution assay (Svoboda *et al.* 1993). The reason for this discrepancy is not known.

CONCLUDING REMARKS

Until recently there was little evidence that the splice variations in $G_{s\alpha}$ have more than marginal significance with respect to their properties and function (Graziano *et al.* 1987, 1989, Mattera *et al.* 1989, O'Donnell *et al.* 1991, Jones *et al.* 1990, Freissmuth *et al.* 1991). In addition, the region of variation between the long and short variants of $G_{s\alpha}$ has been modified by genetic engineering to produce an epitope-tagged variant of $G_{s\alpha}$ which behaves in a similar manner to the wild-type protein (Levis & Bourne 1992).

It is hard to believe, however, that the complicated scheme of alternative splicing and energy-consuming synthesis of proteins from corresponding transcripts would proceed without any functional use. As summarized in this review, abundant data in the literature show that the steady-state mRNA and protein levels of the long and short variants of $G_{s\alpha}$ change dramatically during ontogenetic development, ageing, cellular differentiation, gestation, cold acclimation and pathophysiological states such as obesity, hypertension, diabetes and alcoholism. This descriptive evidence, although indirect and circumstantial, supports the idea that the expression of the two $G_{s\alpha}$ variants is regulated according to the functional requirements of a given cell or tissue.

Similarly, there is no consistent evidence that the changes in the relative proportion of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ regulate effector activity. The increase in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio was found to be associated with an increase (Granneman *et al.* 1990, Chaudry & Granneman 1991, Urasawa *et al.* 1991) as well as a decrease in adenylate cyclase activity (Svoboda *et al.* 1993, Kvapil *et al.* 1995*b*), and the decrease in the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio was accompanied by an increase (Rius *et al.* 1991, Kilgour & Anderson 1993), no change (Michel *et al.* 1994) or a decrease (Green & Johnson 1989, Begin-Heick 1992) in the enzyme activity. More specifically, a dramatic increase in $G_{s\alpha\text{-}S}$, accompanied by little or no change in $G_{s\alpha\text{-}L}$, was found to proceed together with an increase (Walseth *et al.* 1989, Rius *et al.* 1991, Kilgour & Anderson 1993), no change (Michel *et al.* 1994) or a decrease (Green & Johnson 1989) in adenylate cyclase activity. On the one hand this is not surprising, because the final effect of $G_{s\alpha}$ proteins on adenylate cyclase is modulated by other constituents of a complex G-protein-regulated signalling pathway (such as $G_{i\alpha}$ and $G_{\beta\gamma}$, to name just two) but, on the other hand, the lack of correlation between the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio and adenylate cyclase activity might indicate that the functional meaning of alternative splicing of pre-mRNA is not just regulation of coupling *per se* but regulation of some other process(es) not directly involved in the receptor–G-protein–adenylate cyclase–cAMP cascade.

According to such an alternative view, the differential expression of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ would be part of some physiological or pathophysiological 'programme' (differentiation, recruitment, obesity, etc.). Interesting parallels supporting this view may be found in brown and white adipose tissue. In brown adipose tissue from cold-acclimated animals (Svoboda *et al.* 1993, 1996*a*, Kvapil *et al.* 1995*b*), the ratio between the long and short splice variants of $G_{s\alpha}$ protein is increased. During postnatal development of brown adipose tissue, a change in the proportion between these isoforms has also been observed, with a higher $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio being found in 1-week-old pups (high degree of recruitment) and a lower ratio in 1-month-old pups (lower degree of recruitment) (Granneman *et al.* 1990,

Chaudry & Granneman 1991). If the postnatal change is understood as being a response to the environmental conditions (Nedergaard *et al*. 1986, Obregon *et al*. 1989) rather than being an ontogenetic change after birth, then both the perinatal changes and the changes during cold acclimation follow a new general pattern of decreased relative levels of $G_{s\alpha\text{-}S}$ vs $G_{s\alpha\text{-}L}$ in functionally recruited states of brown adipose tissue.

Interesting parallels can be found in white adipose tissue. During differentiation of white adipocytes in culture, the $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio is decreased (Dennis-Henriot *et al*. 1996). Similarly, Green & Johnson (1989) found a higher $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio in white adipocytes from young rats than in those from aged rats, and a higher $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio was observed in controls than in *ob/ob* and *db/db* mice (Begin-Heick 1990, 1992, 1996). Thus, in adipose tissue, a higher degree of 'recruitment' is probably in general associated with a higher $G_{s\alpha\text{-}L}/G_{s\alpha\text{-}S}$ ratio.

A more provocative view is that the changes in G-protein levels during recruitment and/or differentiation should not be seen just as passive events but as causative ones. Such an opinion has been recently promoted especially by Malbon and co-workers, who suggested that the balance between $G_{s\alpha}$ and $G_{i\alpha}$ controls adipogenesis independently of adenylate cyclase and cAMP (Wang & Malbon 1996, Malbon 1997). Related or not, in human atrium, cAMP was found to be an unlikely candidate for regulation of splicing events or post-translational modification of $G_{s\alpha}$ (Monteith *et al*. 1995). Therefore it might be of interest for future experiments to study the regulation of $G_{s\alpha\text{-}L}$ and $G_{s\alpha\text{-}S}$ expression not as a passive event but as a causative factor of physiological functions.

Since different tissues and cells possess different complements of receptor subtypes, adenylate cyclase isoforms and $G_{\beta\gamma}$ combinations with potentially different properties with respect to their interactions with $G_{s\alpha}$ splice variants, preferential coupling of $G_{s\alpha\text{-}L}$ or $G_{s\alpha\text{-}S}$ to certain subtypes of individual components (receptors and adenylate cyclase) of the adenylate cyclase signalling system may help to explain the possible variability in $G_{s\alpha}$ splice variant function. Such a hypothesis, however, remains to be addressed in the future, because no data have been published to date.

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